

# User Manual

## PepStar™ Microarrays - Multiwell

Ready-to-use peptide microarrays for humoral immune response profiling and epitope mapping for 7 or 21 individual samples in parallel

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Please read the entire manual before starting your experiments!  
Carefully note the handling and storage conditions of peptide microarrays.  
For research use only. Not intended for any animal or human therapeutic or diagnostic use.

## 1. Introduction

Antibody-antigen interactions are key events in immunology. Therefore, the identification of epitopes or immunodominant regions in antigens represents an important step in the characterization of antibodies. One of the most efficient ways to identify such epitopes is incubation of a collection of antigen-derived peptides displayed on glass slides (PepStar™ peptide microarrays) with antibodies of interest.

JPT Peptide Technologies' PepStar™ peptide microarrays represent customized or catalog peptide microarray slides covering individual or collections of proteins for rapid screening of protein-peptide interactions. The peptides displayed on glass slides are chemoselectively and covalently bound, enabling effective interaction with binding partners. Immobilized sequences represent overlapping peptides derived from single or multiple antigens as well as epitope and random peptide collections allowing efficient profiling of humoral immune responses using patient samples and protein-protein interaction studies. In addition, full proteome PepStar™ microarrays are available covering overlapping peptide scans through complete microbial or viral proteomes. Upon incubation with your protein or patient sample the binding event can be detected by fluorescently labelled primary or secondary (2nd) antibody.

## 1.1 About PepStar™ Peptide Microarrays

JPT Peptide Technologies' PepStar™ peptide microarrays comprise synthetic peptides, derived from antigens (principle of epitope detection see Figure 1) or other sources that are chemoselectively and covalently immobilized to the glass surface. An optimized hydrophilic linker moiety is inserted between the glass surface and the peptide sequence to avoid false negatives caused by sterical hindrance. For technical reasons all peptides contain a C-terminal glycine.

JPT's PepStar™ peptide microarrays are designed for detecting potential biomarkers for infectious diseases, autoimmune diseases, cancer and allergies and to elucidate protein-protein interactions. Each spot in the microarray represents a single individual peptide.

After incubation of the peptide microarray with an analyte, a fluorescently labelled detection molecule is used for signal readout.

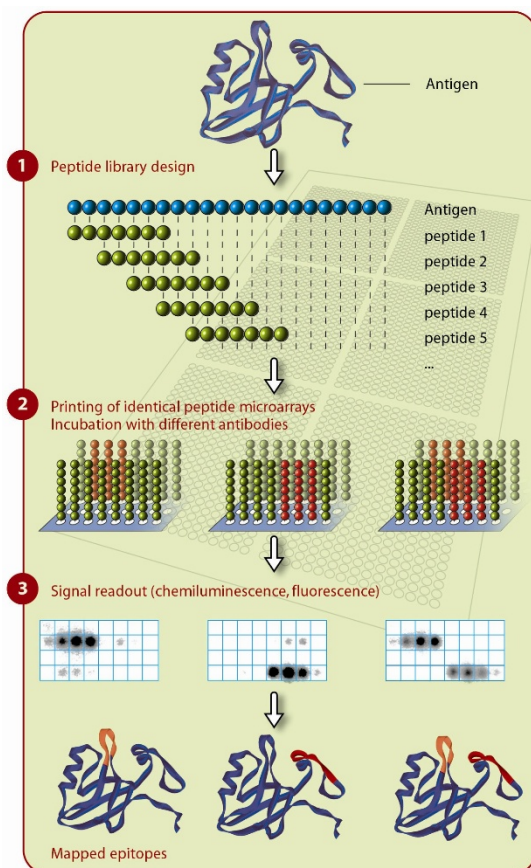


Figure 1: General principle of epitope detection using overlapping peptide scans.

All peptides are displayed in 21 identical subarrays on each slide. PepStar™ slide surfaces are delivered in a pre-treated form minimizing unspecific binding of your target sample. Therefore, usually no blocking step is needed.

## 1.2 PepStar™ Multiwell Peptide Microarray Layout

Please refer to the .gal-file provided with the product documentation for identity and location of the spots on the microarray surface. The microarray side carrying the engraved label represents the surface displaying the attached peptides. The .gal-file can be opened using microarray evaluation software-modules capable of evaluating high-density microarray slides or for simple analysis by JPT's Microarray Feature Viewer. Since .gal-files are tab-separated text files, they can also be accessed by text-processing software such as Microsoft Editor (Notepad) or Microsoft Excel. With the .gal-file provided, evaluation can be performed using software modules like GenePix, ArrayPro or similar programs, which align the .gal-file induced grid onto the resulting image. Additionally, JPT's Microarray feature viewer (part of data files sent by customer support) may be applied for layout visualization, spot identification and feature extraction using any conventional modern web browser. A schematic and exemplary layout of a PepStar™ Multiwell peptide microarray is shown in Figure 3.

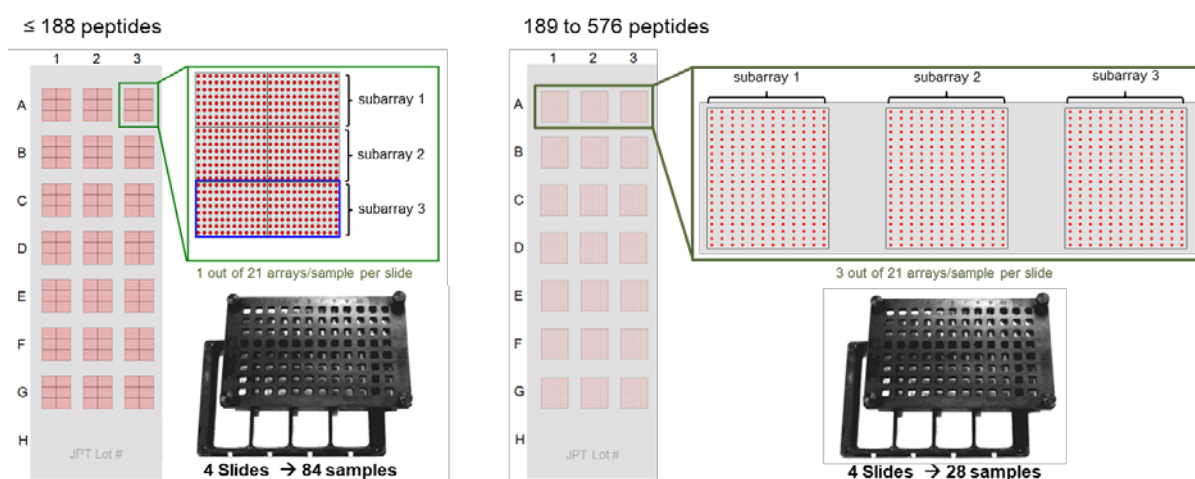


Figure 3: Schematic layout of a PepStar™ Multiwell peptide microarray.

On PepStar™ Multiwell peptide microarrays, all peptides are printed in 21 identical mini-arrays on each slide. Depending on the peptide number each of these mini-arrays contains either three identical subarrays ( $\leq 188$  peptides) or only one set of peptides (189 to a maximum of 576 peptides).

Since JPT recommends the incubation of each sample on three individual sets of the peptide library, either 21 samples (Figure 3 left: 21 mini-arrays with 3 subarrays each) or 7 samples (Figure 3 right: 3 mini-arrays per sample) can be processed in parallel. This enables highly efficient intra-chip-reproducibility tests using scatter plots or correlation functions.

Using the 96-well microarray incubation chamber, depending on the peptide number 84 or 28 samples can be incubated on 4 slides simultaneously (Figure 3). The format of the 96-well microarray incubation chamber corresponds to a standard 96-well plate format and allows therefore the usage of standard ELISA equipment – incubators and washers. Due to the engraved label indicating the unique microarray-lot-number, row H is not utilizable as shown in Figure 3.

## 1.3 Assay Principle

The most common application of JPT's PepStar™ peptide microarrays is the epitope mapping procedure (Figure 1). A schematic view of the assay principle is shown in Figure 2.

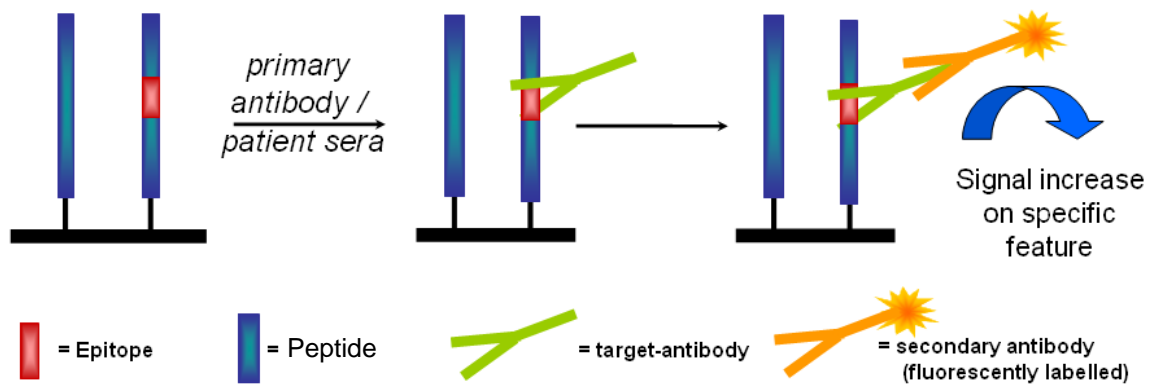


Figure 2: Peptide microarray assay principle.

The peptide microarray is incubated using a primary antibody or patient sample – e.g. serum, plasma or saliva – for a defined time. This enables the formation of stable peptide-antibody interactions. Subsequent to this incubation, the fluorescently labelled secondary antibody is applied. Bound to the peptide-bound primary antibodies, the fluorescence label of the secondary antibody enables readout of antibody interaction by microarray scanning systems. Each spot that shows an interaction with the primary antibody will gain signal in the resulting scan-image.



It is crucial to perform control incubations in order to distinguish between real signals and false positives. To avoid false positive signals induced by peptide-secondary antibody interaction, JPT recommends performing regular control incubations using secondary antibodies only. In addition, JPT recommends performing control incubations applying unrelated primary antibodies to check for false positive signals induced by interaction of peptide with Fc-fragment of the primary antibody.



For seroscreening application JPT recommends checking the secondary antibody for selectivity and specificity. Signals induced by cross-reactivity of secondary antibodies directed against IgG towards IgM or IgE may result in false positive results.

## 1.4 List of Components

Component	Quantity
PepStar™ Multiwell Peptide Microarray	Glass slide displaying peptides in 21 identical mini-arrays. Printed in a pattern suitable for Multiwell-incubation chamber (also available from JPT).
Multiwell incubation chamber (if ordered separately)	Incubation chamber allowing parallel incubation of up to four microarray slides, enabling parallel assay of up to 84 individual samples.
Product Documentation	Relevant data files (protocol and datasheet as pdf-file and sequence info as gal-file and JPT's Microarray Feature Viewer as zipped package).

## 1.5 Storage and Handling

### Storage of Peptide Microarray Slides

- Optimal storage conditions for peptide microarray slides are in a cool (approx. 4°C / 39°F) and dry environment.
- Peptide microarrays are stable for at least 6 months when stored at 4°C (39°F).
- **Do not freeze** the peptide microarrays.

### Handling of Peptide Microarray Slides

- Always handle the peptide microarrays with care.
- Never touch the peptide microarray slide surface.
- Always wear powder-free laboratory gloves when handling peptide microarray slides.
- Hold peptide microarray slides at the end with the engraved data label, with the data label face up. This label provides a unique identification of the specific microarray.
- Take care when dispensing solutions onto the slide surface. Make sure not to touch the surface with pipette-tips or dispensers.
- Inappropriate chemicals may destroy the chemical bonding of the peptides to the glass surface. Never use chemicals with corrosive activity.
- Usage of strong alkaline or acidic solutions should be avoided.
- Avoid dust or other particles during each step of the experiment. Dust, particles and resulting scratches will cause artifacts during the final signal readout.
- Filter all solutions for the washing steps through minimum 0.2 µm particle filters before use (preferably even 0.4µm particle filters).

## 1.6 Additional Material Required

### Additional Reagents

Component	Recommendation & Remarks
Primary antibody	Final concentration of about 1 µg/ml.
Proteins / enzymes	For analysis of e.g. protein binding components, JPT recommends a final concentration of 0.1 µg/ml or above.
Blood sera or plasma solution	Final sample dilution of 1:100 to 1:500 in blocking reagent or assay buffer.
Secondary antibody	<p>Fluorescently labeled secondary antibody.</p> <p>JPT recommends use of conjugates with far-red emitting fluorescent dyes (DyLight 649/AlexFluor 647/Cy5 or similar). Blue and green fluorescence emitting dyes are not recommended due to background issues.</p> <p>Detection antibody should be titrated to determine optimal working dilution providing a maximal signal-to-noise ratio with a minimal background binding. The default working concentration (used by JPT) is 1 µg/ml (for antibody epitope mapping) and 0.1 mg/ml (for screening of antibody responses in sera).</p>
Optional: Labeling Kit for Proteins / Antibodies	For direct labeling of proteins or antibodies JPT recommend to use the following kit: DyLight 650 Microscale Antibody Labeling Kit (ThermoFisher Scientific; 84536). Please follow the instruction of use delivered together with the kit for preparing your sample.
Blocking buffer	For sample dilution JPT recommends usage of Superblock T20 buffer (Thermo/Pierce, #37516) or alternatives like e.g. 3% BSA in 1x TBS-Buffer + 0.1% Tween20 (TBS-T).
Washing buffer	1x TBS-Buffer + 0.1% Tween20 (TBS-T).
De-ionized water	For final washing steps of the microarrays.
Silicone Grease	For the gasket of the 96-well microarray incubation chamber. e.g. Bayer Silicon Grease Baysilone (medium viscosity).

**Additional Hardware & Software**

Component	Recommendation & Remarks
Tweezers (optional)	For handling of all PepStar™ Peptide Microarrays
96-Well Microarray Incubation Chamber	Required for incubation of microarrays with multiple samples.
ELISA/Mircoplate Washer (optional)	Alternatively, the 96-well microarray incubation chamber may be washed manually like a conventional ELISA plate.
Orbital Shaker	For the incubation/shaking of the 96-well microarray incubation chamber.
4-Well Dish (optional)	JPT recommends performing all incubation steps using the 96-well microarray incubation chamber. Alternatively, 4-well dish, microscope slide staining dish or 50 ml-falcon tubes can be used for secondary antibody incubation.
Rocking platform	For all manual incubation and washing steps (Note: Do not shake Microarray-Chip-Sandwiches).
Microarray centrifuge	Or access to a stream of nitrogen to dry the microarray slides.
Fluorescence scanner/imager	Capable of excitation of appropriate fluorophore moiety and with a pixel size of at least 10 μm.
Software	Allowing quantification of the image and the assignment of signal intensities to individual peptides using the enclosed gal-file.

## 2. Experimental Protocols



The following procedure is given as a guideline only. The optimal experimental conditions will vary depending on your sample and instruments and can, therefore, not be predetermined. The optimal experimental conditions must be established by the user. No warranty or guarantee of performance using this procedure with your target antibody or serum can be made or is implied.

The PepStar™ Multiwell peptide microarray is designed as a ready-to-use product to identify epitopes, peptide binders or immunodominant regions in antigens.

Ordinary, there is no need to perform blocking steps on the slide surface prior to incubation with the target sample. However, in case of incubations with patient sera or plasma, JPT recommends to include an additional blocking step prior to incubation with patient sample.

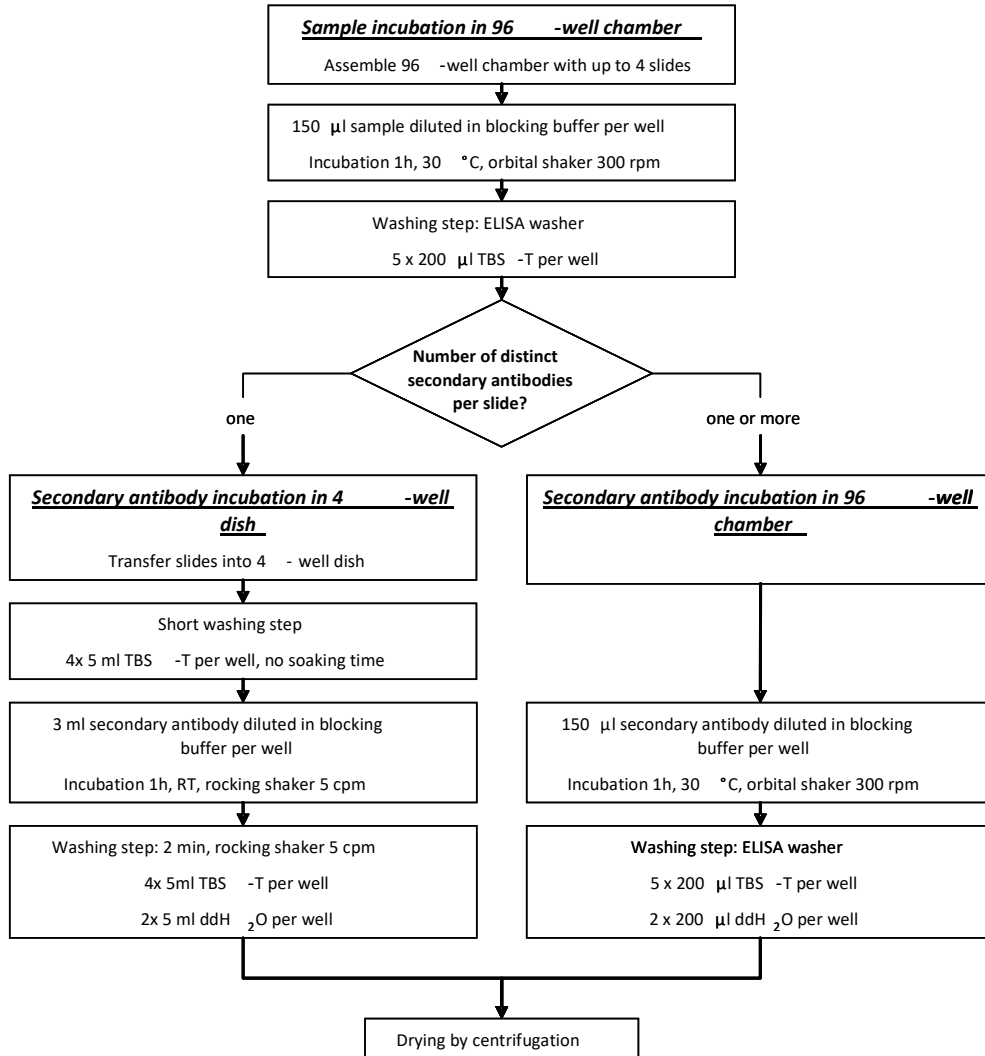
Please refer to the .gal-files for identity and location of the spots on the peptide microarray surface. The side of the slide displaying the peptides is marked with the engraved lot number.

**Note: For analysis of protein/protein-interaction no specific guideline can be provided. Several factors such as buffer components, ion strength, pH-value, temperature, washing conditions and more may influence the binding affinity of the target protein to the immobilized peptides. JPT also recommends to perform a direct labeling reaction of the protein of interest as well as several independent incubations covering different conditions such as concentrations, temperatures and washing procedures.**

### 2.1 Microarray Processing

All PepStar™ Multiwell peptide microarrays produced by JPT have an identical layout concerning active area and spotted surface. Although the content of the microarrays varies, the overall layout and dimensions are the same. All Multiwell peptide microarrays produced by JPT are adjusted to fit into the 96-well microarray incubation chamber allowing parallel processing of 84 or 28 samples using four slides depending on the peptide number (Figure 3).

## 2.2 Workflow of Multiwell Peptide Microarray Incubation



## 2.3 Assay Using 96-well Chamber & 4-well Dish

Assemble 96-well chamber with up to 4 slides. If less than 4 peptide carrying slides are used, please use enclosed blank slides for vacant positions. Only if all 4 slide positions are occupied the chamber will seal the compartments properly.

- 1) Sample incubation
  - 150  $\mu$ l sample diluted in blocking buffer per well
  - 1 h, 30°C, shaking 300 rpm (orbital)
- 2) Washing step using ELISA washer
  - 5x 200  $\mu$ l TBS-T per well

Transfer slides into 4-well dish

- 3) Short washing step
  - 4x 5 ml TBS-T per well, no soaking time
- 4) Secondary antibody incubation
  - 3 ml secondary antibody diluted in blocking buffer per well
  - 1 h, RT, shaking 5 cpm (rocking)
- 5) Washing step
  - 4x 5 ml TBS-T, 2 min soaking, shaking 5 cpm (rocking)
  - 2x 5 ml ddH<sub>2</sub>O, 2 min soaking, shaking 5 cpm (rocking)
    - Do not remove the water after the last washing step: leave the slides in water until drying.
- 6) Drying by centrifugation

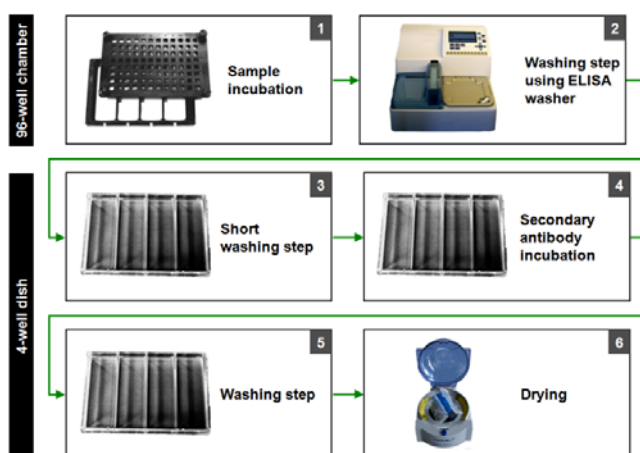


Figure 3: Multiwell array incubation procedure using 96-well chamber and 4-well dish.

**Assay Using 96-well chamber only (recommended)**

Assemble 96-well chamber with 4 slides

- 1) Sample incubation
  - 150  $\mu$ l sample diluted in blocking buffer per well
  - 1 h, 30  $^{\circ}$ C, shaking 300 rpm (orbital)
- 2) Washing step using ELISA washer
  - 5x 200  $\mu$ l TBS-T per well
- 3) Secondary antibody incubation
  - 150  $\mu$ l secondary antibody diluted in blocking buffer per well
  - 1 h, 30  $^{\circ}$ C, shaking 300 rpm (orbital)
- 4) Washing step using ELISA washer
  - 5x 200  $\mu$ l TBS-T per well
  - 2x 200  $\mu$ l ddH<sub>2</sub>O per well
- 5) Drying by centrifugation

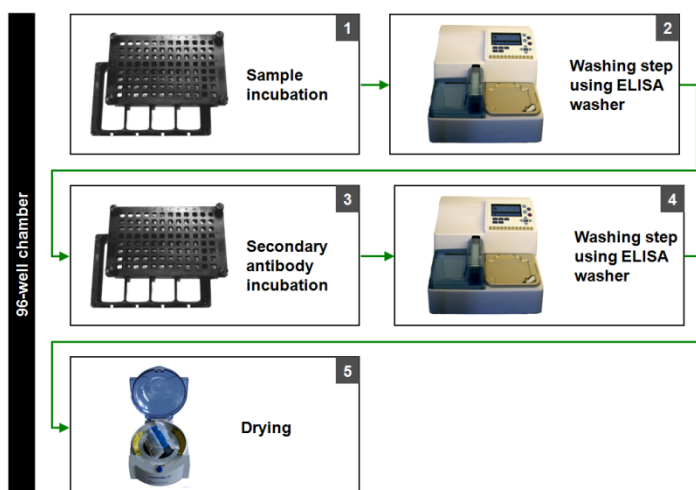
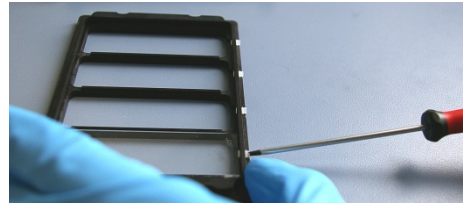
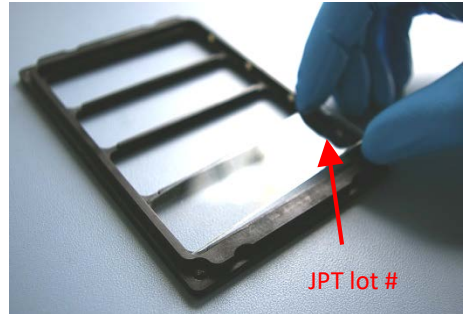


Figure 4: PepStar™ Multiwell array incubation procedure using 96-well chamber only.

**Assembly of 96-Well Chamber**

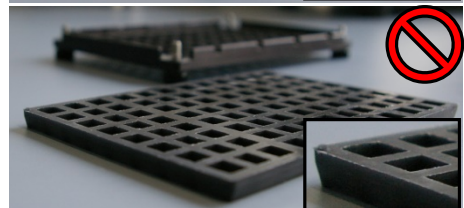
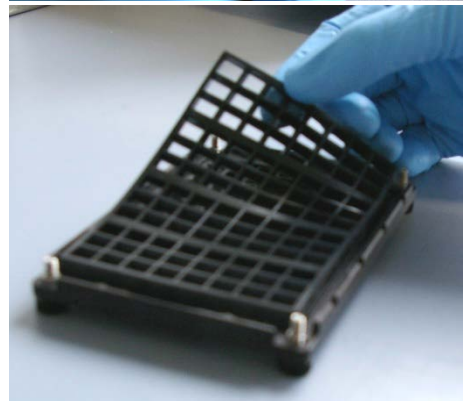
1) Insert the slides into the lower part of the chamber and fix them (one by one).

Orientation: JPT lot number must be readable and placed in row H.



2) Insert the gasket into the upper part of the chamber.

Orientation: Thin side up.

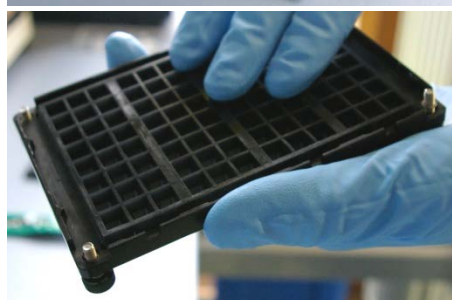
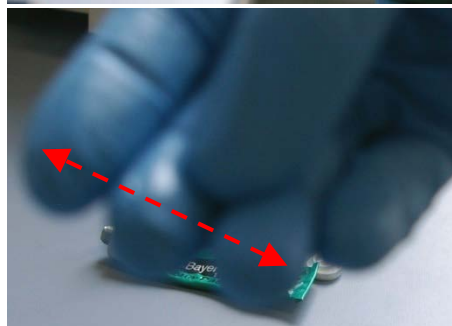


3) Make sure the gasket is sitting properly.

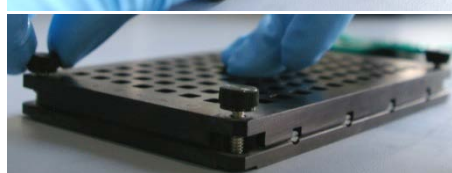


4) Put a **very thin** layer of silicone grease on the gasket.

- Take a tiny amount of silicone grease (less than on the picture).
- Spread it between your fingers to create a thin layer.
- Carefully, apply the silicone grease on the gasket to create a uniform film on all surfaces touching the microarrays.



5) Close and fix the chamber using the four screws.



**Remarks**
**Washing:**

- The settings of ELISA (microplate) washer should be adjusted in advance using standard glass slides. The washing head must not touch the slide surface.
- Alternatively, the 96-well chamber may be washed manually like a conventional ELISA plate.

**Drying:**

- Alternatively to using a slide centrifuge, the slides may be dried by a gentle stream of nitrogen.

### 3. Troubleshooting & Notes

Problem	Cause	Solution
Artifacts	<ul style="list-style-type: none"> <li>• Dust particles and resulting scratches</li> </ul>	<ul style="list-style-type: none"> <li>• Avoid dust or other particles during each step of the experiment</li> <li>• Use filtered buffers and solutions only</li> <li>• When using ELISA washer, increase the height of the washing head.</li> <li>• Reduce the amount of silicone grease used.</li> </ul>
High background	<ul style="list-style-type: none"> <li>• Nature of the sample</li> <li>• Sample / 2<sup>nd</sup> antibody concentration</li> <li>• Insufficient washing</li> </ul>	<ul style="list-style-type: none"> <li>• Direct fluorescently labeled proteins tend to induce background signals via unspecific binding to the slide surface. Changing of buffer conditions in the incubation step can reduce background signals very efficiently</li> <li>• Additional washing steps can reduce non-specific binding</li> <li>• Variation of blocking buffers (initial blocking steps are not recommended by JPT)</li> <li>• Increased concentrations of sample / 2<sup>nd</sup> antibody may yield high background signals caused by unspecific binding to the slide surface</li> <li>• Adjustment of washing conditions</li> </ul>

	<ul style="list-style-type: none"> <li>Contaminated wash buffer</li> </ul>	<ul style="list-style-type: none"> <li>All buffers and solutions should be prepared freshly every day</li> </ul>
Saturated peptide spots	<ul style="list-style-type: none"> <li>Concentration of the 2<sup>nd</sup> antibody</li> <li>Scanning conditions</li> </ul>	<ul style="list-style-type: none"> <li>Higher dilution rates of the 2<sup>nd</sup> antibody</li> <li>Adjustment of scanning parameters</li> </ul>
Unspecific signals	<ul style="list-style-type: none"> <li>Nature of the sample</li> <li>Insufficient washing</li> <li>Specificity of the 2<sup>nd</sup> antibody</li> </ul>	<ul style="list-style-type: none"> <li>Variation of blocking buffers</li> <li>Adjustment of washing conditions</li> <li>Control incubations using labeled 2<sup>nd</sup> antibody alone should be performed in parallel to the actual experiment to ensure that found signals are not caused by non-specific binding of the 2<sup>nd</sup> antibody to the immobilized peptides</li> </ul>
Little or no signals	<ul style="list-style-type: none"> <li>Incubation time</li> <li>Bleaching effects</li> <li>Scanning conditions</li> </ul>	<ul style="list-style-type: none"> <li>Warranty of sufficient incubation time</li> <li>During the incubation step with fluorescently labeled 2<sup>nd</sup> antibody, protect the slides from light!</li> <li>After application of secondary antibody keep slides in an ozone-free environment</li> <li>Adjustment of scanning parameters</li> </ul>

## 4. Contact Us

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Technical Support North America 1.888.JPT.COM0 (1.888.578.2660) <a href="mailto:us-bd@jpt.com">us-bd@jpt.com</a>	

## 5. Product Use & Liability

THE PRODUCTS ARE FOR EXPERIMENTAL LABORATORY USE ONLY AND NOT INTENDED FOR HUMAN OR HOUSEHOLD USE.

Only qualified personnel should handle these chemicals.

Note that missing hazard warnings do not indicate that a product is harmless. Products are for research use only (RUO). JPT Peptide Technologies declines responsibility for any damage arising from the inappropriate use of its products.

JPT Peptide Technologies makes no warranties of any kind, expressed or implied extending beyond the description of the product in this brochure, except that the material will meet our described specifications at the time of delivery.

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